NeoBiota 34: 21–40 (2017)
doi: 10.3897/neobiota.34.10820
http://neobiota.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
NeoBiota
Advancing research on alien species and biological invasions

Biotic constraints on the establishment and performance of native, naturalized, and invasive plants in Pacific Northwest (USA) steppe and forest

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Academic editor: *T. Knight* | Received 17 October 2016 | Accepted 2 February 2017 | Published 23 February 2017

Citation: Connolly BM, Powers J, Mack RN (2017) Biotic constraints on the establishment and performance of native, naturalized, and invasive plants in Pacific Northwest (USA) steppe and forest. NeoBiota 34: 21–40. https://doi.org/10.3897/neobiota.34.10820

Abstract

Factors that cause differential establishment among naturalized, invasive, and native species are inadequately documented, much less often quantified among different communities. We evaluated the effects of seed addition and disturbance (i.e., understory canopy removal) on the establishment and seedling biomass among two naturalized, two invasive, and two native species (1 forb, 1 grass in each group) within steppe and low elevation forest communities in eastern Washington, USA. Establishment within each plant immigrant class was enhanced by seed addition: naturalized species showed the greatest difference in establishment between seed addition and no seed addition plots, native and invasive species establishment also increased following seed addition but not to the same magnitude as naturalized species. Within seed addition plots, understory canopy disturbance resulted in significant increases in plant establishment (regardless of plant immigration class) relative to undisturbed plots and the magnitude of this effect was comparable between steppe and adjacent forest. However, regardless of disturbance treatment fewer invasive plants established in the forest than in the steppe, whereas native and naturalized plant establishment did not differ between the habitats. Individual biomass of naturalized species were consistently greater in disturbed (canopy removed) versus undisturbed control plots and naturalized species were also larger in the steppe than in the forest at the time of harvest. Similar trends in plant size were observed for the native and invasive species, but the differences in biomass for these two immigration classes between disturbance treatments and between habitats were not significant. We found that strong limitations of non-native species is correlated with intact canopy cover within the forest understory, likely driven by the direct or indirect consequences of low light transmittance through the arboreal and understory canopy.

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Considered collectively, our results demonstrate how seed limitation and intact plant ground cover can limit the abundance and performance of naturalized species in Pacific Northwest steppe and low elevation forest, suggesting that local disturbance in both habitats creates microsites for these species to establish and survive. Future studies evaluating interactions between multiple barriers to establishment using more representatives from each immigration class will further reveal how biotic interactions ultimately influence the demography and distribution of non-native plants within these communities.

Keywords
Disturbance, seed limitation, biotic resistance, competition, mesic steppe, coniferous forest, seedling establishment, seedling performance

Introduction

Naturalizations form the small fraction of those introduced species that have surmounted demographic and local environmental barriers to develop self-sustaining populations, but unlike invaders, naturalized species do not inevitably proliferate within the novel habitat (Blackburn et al. 2011, Richardson and Pyšek 2012). Limitation of naturalized species in their abundance and geographic range may result from demographic restrictions, dispersal limitations, abiotic constraints, or trophic interactions in the novel range (Davis 2009, Richardson and Pyšek 2012, Pearson et al. 2012, Connolly et al. 2014). Furthermore, naturalized species often establish more readily and have higher fitness in disturbed habitats (MacDonald and Kotanen 2010, Maron et al. 2012, Maron et al. 2013), suggesting that competition for microsites may be a major determinant of plant naturalization (Going et al. 2009, Kempel et al. 2013). However, despite the role of naturalizations as precursors to invasions, we know surprisingly little about how demographic, physical, and biotic factors interact within a novel range to curb, delay or prevent naturalized species from becoming invasive (Richardson et al. 2000, van Kluenen et al. 2010, Richardson and Pyšek 2012).

The physical and biotic factors governing plant establishment are frequently quantified (e.g., Mack and Pyke 1984, Pyke 1986, Weiher and Keddy 1999, Myers and Harms 2009), but the effect of these factors on the fate of naturalized species compared to the fate of co-occurring invaders is unclear (van Kluenen et al. 2010). We compare and contrast here the effects of two factors – disturbance and seed limitation – on the establishment and subsequent performance of native, naturalized, and invasive species between two community types that differ radically in invasion history. The proliferation of many temperate plant species are limited by seed recruitment (Turnbull et al. 2000, Clark et al. 2007), suggesting low abundance of naturalized species or poor dispersal ability may be related directly to low seed availability. Introduced species also likely differ in their tolerance of highly competitive environments, e.g. the recruitment of naturalized species may be more strongly limited by native canopy cover than by co-occurring invaders. Consequently disturbance by the removal of competitors can differentially influence the establishment

of introduced plants (Gross et al. 2005), but the specific response of invasive vs. naturalized species to this disturbance is unclear.

The potential for a species' immigrants to naturalize and the descendants to invade can also vary by habitat (Rejmanek et al. 2005, Richardson and Pyšek 2012). The dominance of non-native species can vary enormously among habitats in novel ranges, a relationship often largely described as a reflection between introduced plants and response to the climate of their new habitat (Alpert et al. 2000). The availability of microsites and the severity of interspecific competition, however, will be functions of resource availability (Rejmanek et al. 2005, Chytrý et al. 2008), and species naturalized or invasive in resource-rich habitats may be rare or excluded in adjacent habitats that lack critical resources (e.g., Huenneke et al. 1990). For example, low light transmittance through the forest canopy and understory can be a major barrier prohibiting many non-native species, particularly grasses, from invading forests (Pierson and Mack 1990, Brothers and Spingarn 1992, Martin et al. 2009), but shade may not inhibit the establishment of other non-natives (e.g. *Microstegium vimineum*, Martin et al. 2009, Flory 2010). Recruitment of non-native species is often much greater when seed additions can co-occur with disturbance of the forest understory or overstory (Pierson and Mack 1990, Dodson and Felder 2006). To date, however, no comprehensive evaluation has been assembled of the effect of understory canopy disturbance on the concurrent establishment rates and performance of naturalized versus invasive species in forests and adjacent grasslands.

Meadow steppe and adjacent coniferous forest in eastern Washington (USA) have experienced markedly different levels of plant invasion. Non-native grasses and forbs are prevalent in steppe (Daubenmire 1970, Mack 1986) but infrequent in adjacent coniferous forests (Daubenmire and Daubenmire 1968, Parks et al. 2005). When the understory is removed, seedling establishment of some non-native species is not however otherwise limited by differences between these communities (Connolly 2013). Additionally, preferential granivory partially explains differences in the abundance of naturalized and invasive species within the steppe (Connolly et al. 2014) but fails to account for the low abundance of non-native species in these forests. To an un-quantified degree, the realized distribution of native, naturalized, and invasive species within the steppe and forest communities may be a function of seed limitation and the ability for species to persist in undisturbed habitat (Pierson and Mack 1990).

We examined the effect of seed addition and local disturbance (i.e., removal of all plant material <1.5 m above the ground) on the establishment and performance of native, naturalized, and invasive species in meadow-steppe and forest habitats in eastern Washington (USA) as part of a multi-pronged investigation of the forces that restrict/enhance naturalization (Connolly 2013, Connolly et al. 2014). Our objectives were to 1) quantify the severity of seed limitation for a set of representative native, naturalized, and invasive species, 2) evaluate how disturbance of the understory canopy cover influenced recruitment and performance of each class of immigrant, and 3) evaluate the effect of these factors within invaded steppe and uninvaded forests.

Materials and methods

Study sites

A total of eight steppe and forest study sites were chosen that span the meadow steppe-xerophytic forest ecotone in eastern Washington (See Suppl. material 1: Table S1). The co-dominance of *Symphoricarpos albus* with *Festuca idahoensis* and *Pseudoroegneria spicata* characterize the mature vegetation in the *Festuca idahoensis*/*Symphoricarpos albus* habitat type (*sensu* Daubenmire 1970) in the four eastern Washington meadow-steppe sites (1250 m^2 each). The four forest sites (1250 m^2 each) are dominated by *Pinus ponderosa* with co-dominate *Symphoricarpos albus* in the understory (hereafter termed the *P. ponderosa*/*S. albus* habitat type, *sensu* Daubenmire and Daubenmire 1968). Sites were 40.9 ± 6.1 km apart; the adjacent sites were at least > 0.5 km apart.

Study species

A seed mixture of three grasses and three forbs (a native, naturalized, and invasive species of each taxonomic category) was used in seed addition plots in this study. The native perennials *Pseudoroegneria spicata* and *Geum triflorum* are prevalent in meadow-steppe (Daubenmire 1970); these species are less prominent in *P. ponderosa* forests (Daubenmire and Daubenmire 1968). *Secale cereale*, a naturalized annual, is a Washington Class C noxious weed that appears as a volunteer in many cultivated crops (Gaines and Swan 1972, Washington Noxious Weed Control Board [WNWCB]: http://www.nwcb.wa.gov) and establishes, albeit rarely, in meadow steppe and *P. ponderosa* forest (Connolly et al. 2014, USDA PLANTS database: http://plants.usda.gov). *Centaurea cyanus*, a naturalized annual, is also registered on the WNWCB monitor list and is widely established at low density throughout the meadow steppe and *P. ponderosa* forest in eastern Washington (Roche and Talbot 1986, USDA PLANTS database: http://plants.usda.gov). The invasive annual *Bromus tectorum* is abundant, even dominant, in the meadow steppe (Daubenmire 1970, Mack 1981) but infrequent in *P. ponderosa* forest (Daubenmire and Daubenmire 1968, Pierson and Mack 1990). *Cirsium arvense*, an invasive perennial, commonly occurs in anthropogenically disturbed sites and is present in both habitat types (http://www.nwcb.wa.gov, Connolly 2013). Seeds of *G. triflorum*, *Ce. cyanus*, *B. tectorum*, and *Ci. arvense* were collected in bulk from our meadow steppe sites from May – September 2010 and 2011; seeds of *P. spicata* and *S. cereale* were obtained from a local vendor (Rainer Seed Company, Davenport, WA, USA) to insure we had adequate numbers of locally produced seeds for all treatments (described below).

We substantiate the immigrant class (naturalized vs. invasive) of each non-native test species based on 1) a preliminary vegetation analysis conducted at all 8 study sites (Connolly et al. 2014) and 2) state and regional published accounts habitats (e.g., Gaines and Sawn 1972, Roche and Talbot 1986) of the relative abundance of these

species. Importantly, some work has evaluated the mechanisms driving competition dynamics between these specific native perennials and introduced annuals (e.g., Madsen et al. 2012), but outcomes remain unclear and suggest evaluation of their respective establishment potential and relative performance across environmental and disturbance gradients may help identify the drivers of introduced plant colonization and persistence in natural sites.

Two-factor field exclosure experiment in steppe and forest

The effects of seed addition and disturbance were assessed in late July-early August 2011 in six experimental blocks arranged in a 2 × 3 grid at each site (25 × 50 m); blocks were 25-m apart. Each block was comprised of four hardware cloth exclosures (aboveground dimensions were 45 × 45 × 45 cm tall, 1 cm^2 openings); exclosures in each experimental block were arranged 2-m apart in a square (24 exclosures per site, 192 exclosures total across all sites). Before its installation each exclosure was sprayed with enamel paint (Krylon®) to prevent leachate from the hardware cloth affecting plant growth within the exclosure. Exclosures were embedded 15-cm deep into the mineral soil to exclude the treatment being confounded by vertebrate seed predators.

Each block contained a complete 2 × 2 factorial cross with seed addition and disturbance as factors. To generate disturbance treatments, we removed all vegetation and litter from the soil surface and churned the top 3 cm of mineral soil without removing any soil. Disturbed soil was then leveled within each exclosure to minimize differences in soil microtopography among these exclosures (Harper 1977). We extended disturbance treatments in a 0.5-m buffer zone around each disturbance treatment exclosure to minimize shading by neighboring understory plants. Vegetation was left intact within and around undisturbed control exclosures. Exclosures were embedded carefully around each replicate assigned to the undisturbed treatment and produced no detectable changes to plant cover within or around the exclosures. Importantly, this disturbance treatment did not necessarily release experimentally sown plants from competition but rather increased the availability of some resources (e.g., light, Suppl. material 2: Figure S1) that are known to influence plant competition in understory environments.

In early August 2011, 96 exclosures amongst the sites were sown with an admixture of seeds containing three grasses (*P. spicata*, *S. cereale*, *B. tectorum*) and the three forbs (*G. triflorum*, *Ce. cyanus*, *Ci. arvense*). Seeds were sown evenly across a 30 × 30 cm square at the center of each exclosure (0.09 m^2 sampling area, 50 seeds of each species, 300 seeds sown total per exclosure). Seeds were pressed firmly onto the soil surface to minimize post-dispersal seed movement. In the remaining 96 exclosures amongst the sites no seeds were added in order to measure natural recruitment of study species and evaluate the contribution of seed addition to plant establishment counts.

Exclosures were monitored monthly for damage and other extraneous events; plants were counted in early July 2012 to estimate establishment. Following July counts, all above ground plant biomass was harvested within each exclosure, separated

by species, dried (48 hours at 70°C) and weighed. Plant establishment was quantified early in the growing season and before the production of reproductive structures in order to minimize the possibility of introducing non-native species. Natural recruitment by species other than our six test species was rare within these exclosures; nonetheless these recruits were excluded from the analysis. Average individual seedling biomass was estimated by dividing total biomass for each species in each exclosure by the number of that species in the exclosure. Plots that received seed addition were treated with glyphosate herbicide (Roundup®, Monsanto Company) at the cessation of the study. Additionally, the immediate area in a 15-m radius surrounding each exclosure was monitored throughout 2012 and 2013 to detect and remove extraneous introductions.

Statistical analysis

We used general linear mixed models to evaluate whether seed addition, disturbance, and plant immigration class (Native vs. Naturalized vs. Invasive) influenced the number of individuals that established within each community (Steppe vs. Forest). July individual counts of each species were averaged across all blocks at a site to generate site-level averages for each treatment combination and for each species. Ten exclosures were damaged in March 2011. These units were excluded from analysis as vertebrate seed predators and grazers can strongly influence plant establishment in these habitats (Connolly et al. 2014) and may generate undetectable variation in seedling recruitment. Five of eight sites, however, had no damage to exclosures, and no site with damaged exclosures had fewer than three replicates of each treatment combination with which to generate site-level averages for each species. Site-level averages for plant counts for each species were used as model response variables and all fixed effects (habitat, seed addition, disturbance, plant immigration class) and their possible interactions were included in analysis of the response variables. Site identification and the interaction between site, seed addition, and disturbance were included in this model as random effects to account for the nested structure of the design. Average individual counts for each species were log (x+1) transformed prior to analysis. We used *post hoc* tests to evaluate pairwise contrasts using the Tukey-Kramer method to control for multiple comparisons (Littell et al. 2006).

Analysis of average individual biomass followed a similar model structure but was limited to seed addition plots to insure the analysis was conducted between individuals with similar durations of residence time within each plot. Individual biomass estimates of each species were averaged across all blocks at a site to generate site-level averages for each treatment combination and for each species. Individual biomasses were square-root transformed before analysis. Average *Ci. arvense* biomass at one steppe site (Smoot Hill - Summit) was a significant outlier differing from the species' other mean values by over three standard errors and was driven by the rapid second year growth of an adult *Ci. arvense* already residing in the plot. Omitting this observation permits the analysis to satisfy assumptions of normality; consequently, final model analysis for average individual biomass did not include this observation. Models evaluating plant

establishment and biomass employed the Kenward-Roger approximation to estimate appropriate degrees of freedom (Littell et al. 2006). All analyses were conducted in SAS (Proc GLIMMIX, SAS 9.3; Cary, North Carolina, USA).

Our experimental design incorporated the effect of plant immigration class (Native, Naturalized, or Invasive) by evaluating two representative species from each class (one grass, one forb). Although the species selected represent common or dominant plants in these forest and steppe communities (See Study species section) and site-level quality can be assessed by the relative abundance of these native and non-native species (Daubenmire 1970, Mack 1981, Pierson and Mack 1990), we were only able to accommodate two species of each plant immigration status within each plot in our experimental design. Given the limited number of species within each immigration class, we must tentatively interpret conclusions drawn from the main effect immigration class or interactions including immigration class. In order to accommodate interpretation at the species level, we include supplemental results and figures that evaluate plant species as a main effect instead of plant immigration class in the same general linear mixed model framework (Suppl. material 3: Tables S2–S3, Figs S2–S3). Importantly, given the early experimental harvest date and relatively large plot size we assume species sown in our seed mixtures demonstrated independent responses to treatments and had negligible effects on the overall emergence and growth of other species occurring in the same plot. Ancillary analysis using statistical models that helps account for that lack of independence with multivariate responses (i.e., MANOVA general linear models evaluating the response of multiple species sown in the same plot) indicate similar results for main fixed effects to those derived from mixed models (Suppl. material 4: Tables S4–S7).

Results

Not surprisingly seed addition plots had greater recruitment than plots without seed addition, but the magnitude of the positive effects of seed addition varied by habitat and disturbance treatment (Table 1, Fig. 1). The positive effect of seed addition on plant establishment was greater in disturbed plots than undisturbed plots and greater in forest plots than plots in the steppe (Fig. 1). July establishment counts for the four native and naturalized species did not differ significantly between the forest and the steppe (Table 1, Fig. 2A, Native spp.: t = -0.93, d.f. = 106.4, $P = 0.937$; Naturalized spp.: t = 0.35, d.f. = 106.4, $P = 0.999$), but fewer individuals of the two invasive species had established in forest than in steppe by July 2012 (Fig. 2A, t = -4.26, d.f. = 106.4, $P = 0.001$). Recruitment of the two naturalized species was almost entirely driven by experimental seed additions (Table 1, Fig. 2B; Naturalized spp.: t = 11.76, d.f. = 161.9, $P < 0.001$). Seed additions also resulted in greater establishment for native species and invasive species relative to plots that did not receive seeds (Native spp.: t = 7.06, d.f. = 161.9, $P < 0.001$; Invasive spp.: t = 4.69, d.f. = 161.9, $P < 0.001$). As of July 2012, the magnitude of the effect of seed addition was greatest for naturalized species, had an intermediate effect on native species, and contributed the least to invasive plant establishment (Table 1, Fig. 2B).

Table 1. General linear mixed model analysis describing the influence of habitat, disturbance, seed addition, plant introduction class, and all possible interactions of these fixed factors on the log-transformed individual counts of plots established in Pacific Northwest steppe and forest communities. Significant differences at a Type I Error = 0.05 are indicated in bold; marginally significant differences at Type I Error = 0.10 are indicated in italics.

Factor	log (Plant Number + 1)		
	F	**d.f.**	***P***
Habitat (H)	15.77	1, 6	**0.007**
Disturbance (D)	35.11	1, 18	**<0.001**
Seed Addition (SA)	598.78	1, 18	**<0.001**
H × D	19.93	1, 18	**<0.001**
H × SA	27.40	1, 18	**<0.001**
D × SA	98.89	1, 18	**<0.001**
H × D × SA	4.15	1, 18	*0.057*
Introduction Class (IC)	0.12	2, 144	0.885
IC × H	4.54	2, 144	**0.012**
IC × D	1.36	2, 144	0.261
IC × SA	9.62	2, 144	**<0.001**
IC × H × D	0.62	2, 144	0.542
IC × SA × H	1.47	2, 144	0.234
IC × SA × D	0.11	2, 144	0.892
IC × H × D × SA	0.03	2, 144	0.968

Individual plant biomass was influenced by a significant interaction between plant immigration class and habitat and a marginally significantly interaction between plant immigration class and disturbance treatment (Table 2). Regardless of habitat or disturbance treatment, naturalized species were significantly larger than either the invasive or native species (Table 2, Fig. 3A, B), reflecting important differences in life history between the species in each plant immigration class. Plants were typically larger in the steppe than in the forest (Table 2), but only the two naturalized species displayed a significant difference in average individual biomass between the two habitats (Fig. 3A, Naturalized spp.: $t = -4.93$, d.f. = 24.9, $P < 0.001$). Similarly, plants were typically larger in experimentally disturbed plots than undisturbed plots (Table 2), but only the two naturalized species demonstrated a significant difference in average individual biomass between the two disturbance treatments (Fig. 3B, Naturalized spp.: $t = 4.58$, d.f. = 76.5, $P < 0.001$).

Discussion

Our goal was to determine whether seed limitation and disturbance via canopy removal differentially influence the recruitment and performance of native, naturalized, and invasive species in communities (meadow steppe and coniferous forest) that differ

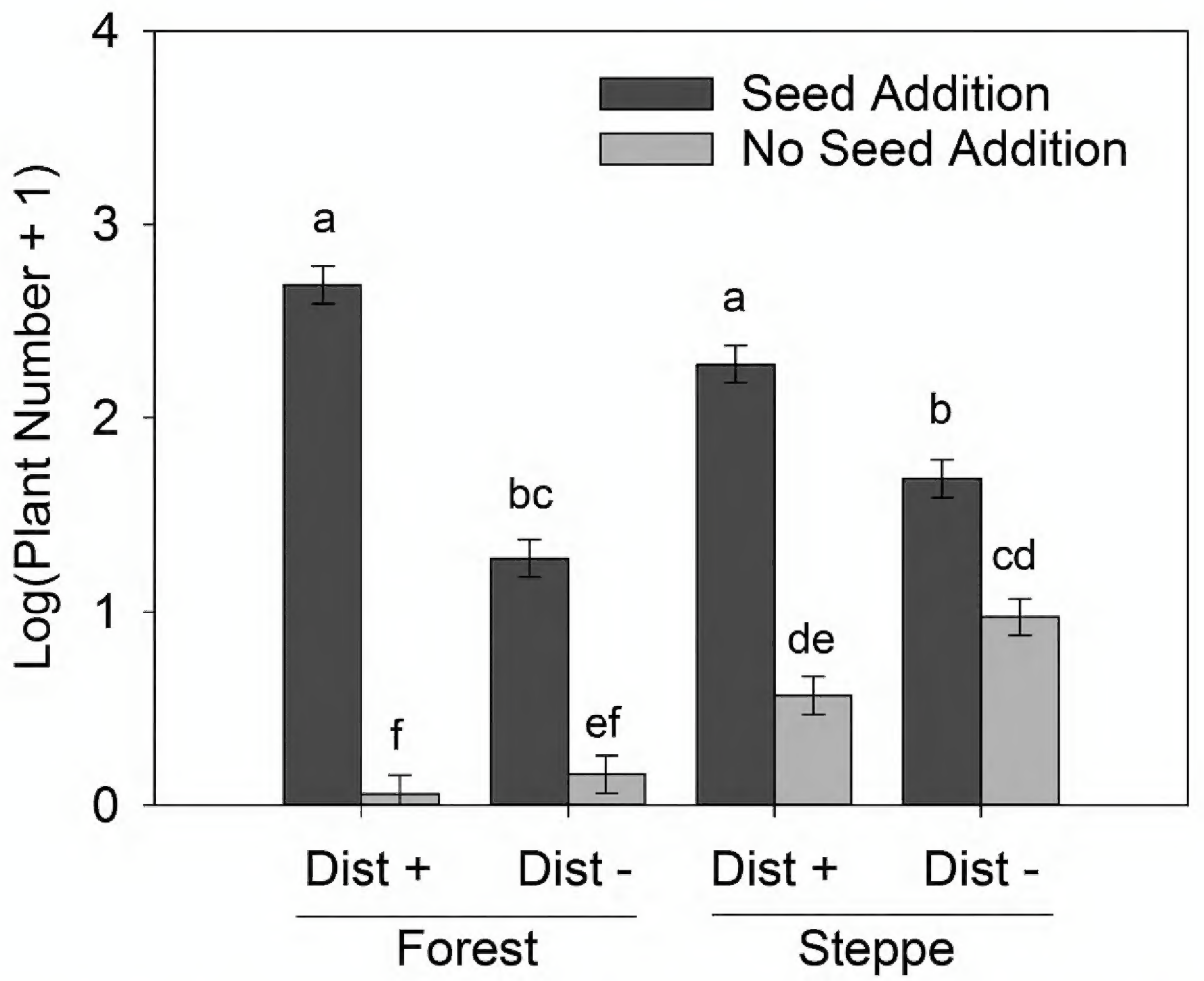

Figure 1. Effect of habitat (Forest versus Steppe), disturbance (Disturbed ["Dist+"] versus Undisturbed ["Dist-"]), and seed addition (Seed Addition versus No Seed Addition) on the average number of total plants in each plot in July 2012. All plant counts are log(x+1) transformed and responses are reported as least square means estimates ± SE. Post hoc multiple pairwise comparisons were conducted with the Tukey-Kramer method; different lowercase letters indicate significant differences at a Type I error = 0.05.

Table 2. General linear mixed model analysis describing the influence of habitat, disturbance, plant introduction class, and the interaction of these fixed factors on the square root-transformed individual biomass of plants harvested (July 2012) from plots established in Pacific Northwest steppe and forest communities. Significant differences at Type I Error = 0.05 indicated in bold; marginally significant differences at Type I Error = 0.10 are indicated in italics.

Factor	√Individual biomass		
	F	**d.f.**	***P***
Habitat (H)	13.96	1, 6.0	**0.010**
Disturbance (D)	125.17	1, 6.2	**<0.001**
Introduction Class (IC)	77.61	2, 72.2	**<0.001**
H × D	0.52	1, 6.2	0.498
IC × H	5.41	2, 72.2	**0.007**
IC × D	2.46	2, 72.2	*0.093*
IC × H × D	0.07	2, 72.2	0.931

radically in physiognomy. Seed limitation differed among the three class with naturalized species the most seed limited, native species intermediately limited, and invaders experiencing intermediate to no limitation. We found that intact plant cover restricts

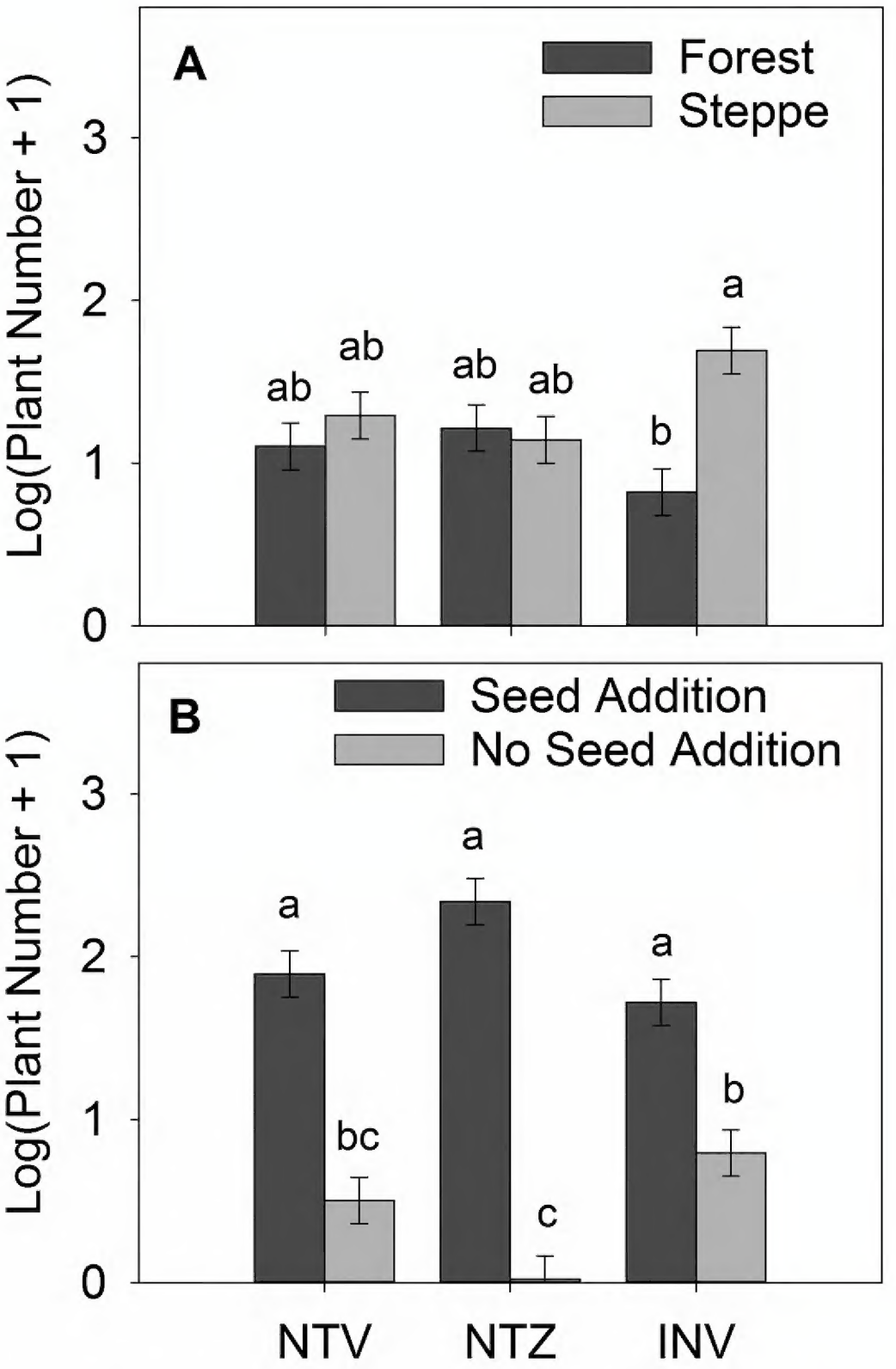

Figure 2. Effect of habitat (**A**) [Forest versus Steppe] and seed addition (**B**) [Seed Addition versus No Seed Addition] on the average number of native ("NTV"), naturalized ("NTZ"), and invasive ("INV") plants in each plot in July 2012. All plant counts are log(x+1) transformed and responses are reported as least square means estimates ± SE. Post hoc multiple pairwise comparisons were conducted with the Tukey-Kramer method; different lowercase letters indicate significant differences at a Type I error = 0.05.

seedling establishment similarly across all plant immigrant class and also results in significantly lower naturalized species growth. Low abundance among naturalized species in PNW meadow steppe and low recruitment of most non-native species in the forest understory are at least partially attributable to the combined influence of seed limitation and low resource availability mitigated by understory canopy cover (e.g., light levels at the soil surface, Suppl. material 2: Fig. S1). Our results, considered simultane-

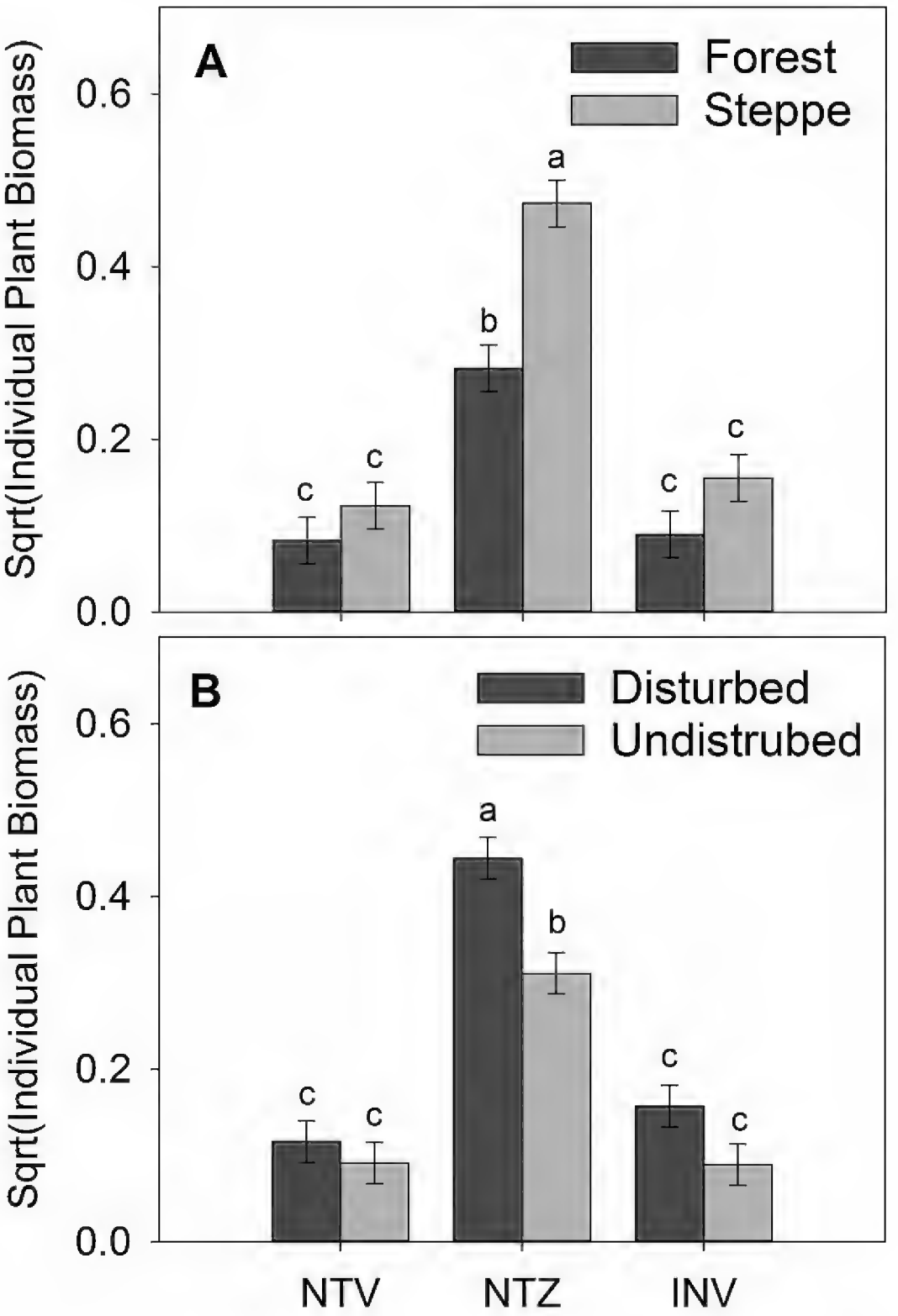

Figure 3. Effect of habitat (**A**) [Forest versus Steppe] and disturbance (**B** (Disturbed ["Dist+"] versus Undisturbed ["Dist-"]) on the average individual biomass of native ("NTV"), naturalized ("NTZ"), and invasive ("INV") plants in each plot as of July 2012. Individual plant biomass estimates were square root-transformed prior to analysis and responses are reported as least square means estimates ± SE. Values are derived solely from seed addition (SA+) plots to insure comparisons were conducted between individuals with similar durations of residence time within each plot. Post hoc multiple pairwise comparisons were conducted with the Tukey-Kramer method; different lowercase letters indicate significant differences at a Type I error = 0.05.

ously with the conclusions of other contemporary studies conducted at these same sites with the same test species (Connolly 2013, Connolly et al. 2014), suggest that biotic resistance can play a major role in determining non-native species abundance (naturalized vs. invasive) within and between these PNW plant communities.

Seed limitation, disturbance, and naturalizations

Seed limitation influences recruitment of many native (Turnbull et al. 2000, Clark et al. 2007) and non-native species (Jongejans et al. 2007, Swope and Parker 2010, Connolly et al. 2014). Seed limitation can be the product of 1) a paucity of reproducing plants, 2) poor seed dispersal, 3) biotic agents that directly reduce seed number, 4) poor propagule viability, or 5) some combination thereof (Harper 1977, Seabloom et al. 2003, Davis 2009). Non-native plants are unlikely to be dispersal-limited between communities in our study region as the propagules of non-native species can readily traverse the PNW steppe-forest ecotone and establish (albeit rarely and for short durations) in disturbed coniferous forest sites (e.g., Pierson and Mack 1990, Dodson and Felder 2006). Unlike native and invasive species, adult *S. cereale* and *C. cyanus* are however rare at both forest and steppe sites (Connolly et al. 2014), implicating the lack of reproducing plants, poor seed dispersal, or both as a major limiting factor for naturalized species within these communities. Moreover, preferential attack by granviores and consistent losses caused by pathogenic soil fungi in both habitats also contribute substantially to seed limitation, occasionally eliminating entire experimentally-introduced populations (Connolly 2013, Connolly et al. 2014).

Differences in species' biomass production between habitat types and with or without disturbance may also influence non-native propagule pressure and contribute to seed limitation for non-native species. For example, individual *B. tectorum* biomass correlates strongly with total seed mass produced per individual plant (R^2_{adj} = 0.861; $P < 0.001$, Almquist 2013) and our study shows average *B. tectorum* biomass was quantitatively greater in undisturbed steppe (43.4 ± 8.2 mg [mean ± SE]) than in undisturbed forest (10.0 ± 4.0 mg) at the time of July 2012 harvest (Suppl. material 3: Fig. S3) suggesting that average annual seed production per *B. tectorum* individual is likely greater in the PNW meadow steppe than in the adjacent, undisturbed ponderosa pine understory. Plants were harvested from exclosures before the generation of reproductive tillers to eliminate unintentional plant introductions at these sites, but previous estimates of *B. tectorum* fitness within each of these communities corroborate this hypothesis (steppe: 16–20 seeds per adult plant, Pyke 1986; forest: 0.7–0.9 seeds per adult plant, Pierson and Mack 1990). Additionally, disturbance of plant canopy cover in our study resulted in 87.5% and 31.7% greater individual *B. tectorum* biomass in the forest and steppe, respectively (Suppl. material 3: Fig. S3). These disturbance-mediated effects on productivity may also increase individual seed production for non-native plants. By limiting productivity, plant cover likely limits non-native plant seed production, influences seed dispersal dynamics, lowers propagule pressure, and facilitates community resistance to the establishment of light-requiring non-native plants.

Disturbance can facilitate a species' transition from naturalization to invasion (Crooks and Soulé 1999, Groves 2006, Chakraborty and Li 2010, Richardson and Pyšek 2012) by increasing resource availability and eliminating competitors (Davis et al. 2000, Davis and Pelsor 2001, Myers and Harms 2009, Richardson and Pyšek 2012, Leffler et al. 2016). In our study, naturalized species' establishment in disturbed

plots in the forest and steppe were equivalent to or exceeded the establishment of co-occurring invasive and native species in identical treatments (Suppl. material 3: Fig. S2), suggesting that resources provided by the removal of understory (< 1.5m high) canopies (e.g., light [Fig. S1], soil nutrients, water) helped meet a major requirement for recruitment for these naturalized species. Seedlings of invasive species may have higher relative growth rates and net assimilation rates than introduced, non-invasive congeners (Grotkopp et al. 2010) and, consequently, invaders may be more robust in resource scarce (e.g., undisturbed) sites than co-occurring naturalized species. Disturbance has a strong, positive effect on the growth of these two naturalized species, suggesting resource limitation, and in particular light limitation, may be a consistent, effective biotic barrier against some members of this class of plant immigrants in PNW forests. Residence time, however, can also influence the potential for naturalized species to invade (Groves 2006) and, while the two naturalized species examined in this study have likely occupied PNW natural habitats for over 100 years (e.g., Gaines and Sawn 1972, Roche and Talbot 1986), it is possible that sufficient time has not elapsed to permit the expansion of these species within these habitats. Further research is needed to determine the extent to which the interaction of resource availability, disturbance regimes, and species residence time in a novel habitat affects the differential establishment of invasive and naturalized species (Grotkopp et al 2002, Groves 2006, Moravcová et al. 2010).

Competition in PNW coniferous forests

Competition in the PNW coniferous forest understory is a strong biotic barrier to invasive species that are abundant in the adjacent steppe, particularly *B. tectorum* (Pierson and Mack 1990). For example, low light availability at the soil surface in the *P. ponderosa* forest understory may cause low non-native species recruitment and individual seedling biomass. *P. ponderosa* forest understory lowered light transmittance at the soil surface to 20% of ambient conditions in June 2012, whereas shading in undisturbed steppe only lowered light transmittance to 60% of ambient conditions at the soil surface (see Suppl. material 2: Methods S1 and Fig. S1). Shading may directly influence the survival of some invasive species; for example, Bakker (1960) reported large *Ci. arvense* seedling mortality if light intensities fall below 20% of full sunlight – a threshold similar to that measured beneath the understory at our Ponderosa Pine forest sites. Additionally, shade lowers the probability that non-native seeds receive essential light-related germination cues (Pons 2000, Jensen and Gutekunst 2003) and may slow non-native seedling growth rate and result in lower fecundity through modifications of seedling microclimate (e.g. low temperatures, increased snow cover, Mack and Pyke 1984, Pierson and Mack 1990).

The environmental tolerances of introduced species interact with a novel habitat to determine a species' potential for naturalization (Richardson and Pyšek 2012), and climatic mismatch between an invader and a novel habitat may preclude non-native

plant establishment (Alpert et al. 2000). Consequently, pre-adaptation to forest understories will raise the likelihood that an introduced species will naturalize in the interior of these temperate North American forests. Shade-tolerant non-native perennials (e.g. *Berberis thunbergii, Celastrus orbiculatus, Lonicera* spp.) readily establish in eastern North American forests (Zheng et al. 2006) and could plausibly be introduced as horticultural escapes and even naturalized in western coniferous forests (Smith and Mack 2013). Some non-native grasses may also tolerate low light levels in North American forest understories and may be candidates for future naturalizations and potential invasions (e.g. *Miscanthus sinensis*, Horton et al. 2010; leptomorphic bamboos, Smith and Mack 2013). Understanding the interactions between the physical tolerances of introduced species and the severity of competition in novel habitats would improve predictions of non-native plant naturalization or invasion potential on a habitat-specific level (Chytrý et al. 2008, Richardson and Pyšek 2012).

Conclusions and future directions

Few studies directly evaluate the relationship between biotic resistance and the relative abundance of introduced species (van Kleunen et al. 2010, Richardson and Pyšek 2012). However, here we report the results of one part of a three-experiment series evaluating how functionally different components of biotic resistance (i.e., seed predation [Connolly et al. 2014], seed parasitism [Connolly 2013], competition [reported here]) relate to the prevalence of non-native plants between habitats differing in susceptibility to invasion. Invasive plants are conspicuous by their tolerance or avoidance, or both, of most biotic barriers in the extensively invaded PNW steppe (Mack 1986), whereas naturalized species are significantly restricted, and occasionally eliminated, by the joint action of biotic interactions in the same habitat and at the same time. In the examples investigated here, community resistance to invasions is substantial in adjacent low-elevation PNW coniferous forest. For the species we evaluated, limitations to recruitment and performance imposed by a dense canopy and seed limitation imposed by granivores and, to a lesser extent, seed pathogens ensure that undisturbed forests interiors are likely to be well defended against the encroachment of many non-native species, particularly annual grasses. Collectively, our work demonstrates that biotic resistance likely plays a role both in determining 1) the distribution of some non-native species amongst a region's communities and 2) the position of a non-native species along the introduced-naturalized-invasive species continuum in a community. Further work evaluating the potential synergistic interactions between multiple biotic barriers with a larger suite of representatives from each immigration class (e.g., Suwa and Louda 2011, Maron et al. 2012, Maron et al. 2013) will help elucidate how biotic interactions ultimately influence demography of non-native plants and the distribution of non-native plants within Pacific Northwest steppe and forest communities.

Acknowledgments

We thank two anonymous reviewers and T. Knight for constructive feedback on the submitted versions of this manuscript. We thank M. Dybdahl, D. Pearson, and L. Carris for helpful comments on earlier drafts of this manuscript. We thank D. Savage and the WSU technical services instrument shop for assistance with exclosure construction. We thank M. Rule for assistance with site identification and permit preparation at the Turnbull National Wildlife Refuge. The Betty Higginbotham trust at Washington State University provided funding to support this work, and AFRI-NIFA Fellowship grant #2014-02074 awarded to B. Connolly provided support while writing this manuscript.

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Supplementary material 1

Table S1

Authors: Brian M. Connolly, Jennifer Powers, Richard N. Mack

Data type: distribution data

Explanation note: Site sites with UTM coordinates and elevation a.s.l.

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Supplementary material 2

Methods S1 and Figure S1

Authors: Brian M. Connolly, Jennifer Powers, Richard N. Mack

Data type: statistical data

Explanation note: Protocol and results figure summarizing light transmittance in disturbed vs. undisturbed plots in PNW steppe and forest.

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Supplementary material 3

Table S2–S3, Figures S2–S3

Authors: Brian M. Connolly, Jennifer Powers, Richard N. Mack

Data type: statistical data

Explanation note: Generalized mixed model output and summary graphs using species, rather than plant immigration class, as a fixed factor in models assessing plant establishment counts and individual plant growth.

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Supplementary material 4

Table S4–S7

Authors: Brian M. Connolly, Jennifer Powers, Richard N. Mack

Data type: statistical data

Explanation note: MANOVA GLM results for seedling establishment and performance measures.

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